Reduction of Natural Ferric Iron Chelators in Disturbed Forest Soils[1]

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ABSTRACT

Hydroxymate siderophores (HS) are high affinity FE^{3+} chelators which are produced and secreted into the soil by various soil microflora, including mycorrhizal fungi. HS are important in the iron nutrition of both the microorganisms which produce them and higher plants, and they may also protect higher plants from soil-borne pathogens. We used a standard bioassay technique to compare HS concentrations in soils from 10 pairs of logged and adjacent unlogged sites in various areas of Oregon. In 8 of the 10 sites HS concentrations were significantly lower in soils from logged areas than in those from adjacent undisturbed forests. HS reductions were greater where slash was burned than where it was unburned or windrowed. Pot tests of soils from one site indicated that Douglas-fir seedlings (*Pseudotsuga menziesii* [Mirb.] Franco) were iron limited in logged and burned but not in undisturbed forest soils. Soil pasteurization induced iron limitation in undisturbed soils, which suggests that iron limitation in soils from the logged and burned area may have been due to reduction of HS-producing organisms.

Additional Index Words: **hydroxymate siderophores, broadcast burning, nutrient cycling, Douglas-fir.**

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IRON, AN ELEMENT ESSENTIAL for all organisms, often occurs in highly insoluble forms within the soil (Emery, 1982). Its availability to plants and soil microorganisms is enhanced by various biologically-produced chelating agents. One important group of chelating agents are the siderophores (from the Greek *sideros*, for iron, and *phoros*, for carrier), low-molecular-weight peptide derivatives with extremely high stability constants for Fe^{3+}. Emery (1982) reports that ferrichrome, a common siderophore, will pull Fe^{3+} from Pyrex glassware or stainless steel. Siderophores are produced and excreted by almost all aerobic and facultative aerobic microbial species that have been studied (Neilands, 1977). Two classes of siderophores are distinguished by the chemical group that binds the iron: catechols, produced by bacteria; and hydroxymates, also produced by bacteria but primarily by fungi (Powell et al., 1982). Microorganisms generally produce siderophores only when they are iron limited, and there is little question that these chelating agents are crucial to the iron nutrition of microbes (Neilands, 1973, 1977; Emery, 1982). Although production has not as yet been detected in higher plants, siderophores are produced by mycorrhizal fungi, and recent studies indicate that they may facilitate iron uptake by higher plants (Powell et al., 1982) and perhaps confer resistance to root pathogens (Kloepper et al., 1980).

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Hydroxymate siderophores (HS), produced primarily by fungi, are ubiquitous in undisturbed soils where their concentration is positively correlated with organic material, clay content, and rooting activity (Powell et al., 1980, 1982; Szaniszlo et al., 1981). Our objectives in this study were to investigate (i) the effect of clearcutting and various kinds of site preparation on soil HS concentrations in a wide variety of sites, and (ii) the effect of alteration of soil HS concentrations on growth of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) seedlings.

MATERIALS AND METHODS

Soil Collection

We collected 1 kg of soil (0–15 cm) from three randomly chosen points within clearcuts and adjacent undisturbed forests at 10 sites (Table 1) in southwest, west-central, and east-central Oregon in the summers of 1981 (southwest sites 1, 2, 3) and 1982 (all other sites). The four southwest Oregon sites, on the east slopes of the Klamath Mountains, are in the mixed conifer forest zone (Franklin and Dyrness, 1973), although the predominant species is Douglas-fir. Soils are Haplohumults. Bedrock composition is highly diverse: substrates are granitic on site 1, marine sedimentary on site 2, and metavolcanic on sites 3 and 4. The two west-central Oregon sites are at relatively low elevations in the *Tsuga heterophylla* zone of the western Cascade Mountains (Franklin and Dyrness, 1973). The predominant tree species is again Douglas-fir. Soils are Xerumbrepts formed from andesitic bedrock. The four east-central Oregon sites are located on the east slopes of the Cascade Mountains in what appears to be a transition between the *Abies grandis* and *Pinus ponderosa* forest zones (Franklin and Dyrness, 1973). Predominant tree species are Douglas-fir and ponderosa pine (*Pinus ponderosa* Dougl. ex Laws). Soils are Vitrandepts formed from pumice overlaying bedrock at variable depths.

Where possible, samples were taken from both broadcast burned, and unburned portions of clearcuts; thus we assayed three disturbance classes: undisturbed, logged and broadcast-burned, and logged-unburned. On southwest Oregon sites 1, 2, 3, and on west-central Oregon site 2, unburned portions of clearcuts had been purposely or accidentally missed during broadcast burning and were easily identified by lack of charcoal and scorching on stumps. On east-central

Table 1—Description of study sites.

Site locale	Elevation, m	Forest zone (Franklin and Dyrness, 1973)	Years since disturbance
Southwest Oregon			
Siskiyou National Forest			
Site 1	400	Mixed conifer	12
Site 2	1700		10
Site 3	1390		10
Site 4	850		2
West-central Oregon			
Willamette National Forest			
Site 1	500	*Tsuga*	3
Site 2	800	*heterophylla*	3
East-central Oregon			
Deschutes National Forest			
Site 1	1300	*Abies grandis-*	2
Site 2	1480	*Pinus ponderosa*	2
Site 3	1700	(Transition)	1
Site 4	1420		2

Oregon sites 1 and 4, unburned portions had been tractor-piled and samples were taken between piles. On other sites, no unburned areas were large enough (approximately 100 m^2 or greater) to eliminate edge effects from adjacent burned areas, and so we sampled only burned areas.

Replicate soil samples from each of southwest Oregon sites 1, 2, and 3 were inadvertently pooled, which gave one sample per site per treatment; therefore for statistical analyses, the three sites were treated as a single group, and the pooled single sample from each site was treated as a replicate for the group. Replicate soil samples from other sites were not pooled, and therefore site-specific means and standard errors are given.

Soils were mixed 1:1 (vol/vol) with distilled, deionized water, held at room temperature for 24 h; and then extracted for 24 h by gravity and vacuum filtration. The extracts were freeze-dried and stored in a dessicator until needed.

Soil Analysis

Freeze-dried soil samples were mixed with 5 mL distilled, deionized water and autoclaved for 20 min. The standard procedure for determining concentration of HS in a soil sample is to grow the bacterium *Arthrobacter flavescens* JG-9, a mutant incapable of producing its own siderophore, on sample extracts. Growth of the bacterium is correlated with the amount of HS in the extract. In order to quantify the concentration of HS associated with a given amount of bacterial growth, *A. flavescens* JG-9 is also grown on media containing a known amount of the Fe^{3+} chelator Desferal (methane sulfonate of iron-free ferrioxamine B; Ciba Pharmaceutical Co.; Summit, NJ). Standard curves are then produced relating diameter growth of *A. flavescens* JG-9 to concentration of Desferal in the growth media. From these standard curves, diameter growth of *A. flavescens* JG-9 on a given soil extract can be associated with a given Desferal concentration, and HS concentration in the unknown sample is then reported as the Desferal equivalent.

We obtained *Arthrobacter flavescens* JG-9 from the American Type Culture Collection, Parkville, MD and maintained the bacterial colonies on the medium described by Estep et al. (1975) with 100 μg Desferal. JG-9 was depleted of siderophore by inoculating a loopful from the slant into 20 mL of the medium minus agar and Desferal and was then grown on a shaker for 48 h. The cultures were diluted in 100 mL sterile distilled, deionized water, and petri dishes containing 25 mL of the agar medium, minus Desferal, were inoculated with 1 mL of this suspension. Desferal standards were made by dilution of a known weight to the concentrations 0.1 μg/mL, 1.0 μg/mL, 10 μg/mL, 100 μg/mL, and 1,000 μg/mL. After autoclaving for 20 min, both standards and soil extracts were absorbed into sterile, rinsed, 13-mm filter pads; placed directly on top of the cooled, solidified, seeded JG-9 plates; and incubated at 26°C. After 48 h, growth was determined by measuring the diameter of the zone of JG-9 growing from the filter pad. Diameter of JG-9 grown on Deseral standards was linearly related to the log concentration of Desferal ($r^2 > 0.99$).

Soil pH was determined from a 1:2 paste (20 g soil to 40 mL water). Soil carbon was determined by combustion and analysis on a LECO Carbon Analyzer after grinding and screening soil through 32-mesh. Nitrogen was measured by semimicro-Kjeldahl digestion ($Se/CuSO_4$ catalyst) followed by ammonium analysis (Technicon Industrial Systems, 1976). Carbon, nitrogen, and pH characteristics of soils tested for HS concentration are shown in Table 2 for each study site.

Pot Tests

Douglas-fir seeds were surface sterilized in 30% H_2O_2 for 1 h, rinsed in sterile, distilled water, and stratified in sterile potato dextrose agar for 30 d at 5°C. Sterile seeds sown in nonsterile or steam-pasteurized, undisturbed forest or adjacent broadcast-burned soils from southwest Oregon site 3 received one of two treatments: control (soil only) or plus-chelated iron (3 mg Sequestrine EDTA per seedling). Five replications per treatment combination, each consisting of one seedling in a leach tube 165 mm deep and 25 mm wide, were completely randomized in a growth room with 16 h daylight and a constant temperature (25°C ± 2°C). Seedlings were grown for 12 weeks before being harvested. The total number of root tips and mycorrhizal root tips were counted through a dissecting microscope, and tops and roots were oven dried and weighed.

Statistical Analysis

Siderophore concentrations in soils were compared among disturbance classes on a given site by *t*-test. Seedling weights, total number of root tips, and number of mycorrhizal root tips were analyzed by a fixed-effects-model analysis of variance, from which least significant differences (LSD) were calculated at the 0.05 level for comparing individual means.

RESULTS

In southwest and east-central Oregon sites, HS concentrations in logged and burned soils ranged from 12% to 47% (average 23%) of those in adjacent undisturbed soils (Table 3). HS concentrations were also reduced in logged and unburned soils of the southwest Oregon sites but not of the east-central sites. In contrast, HS concentrations in logged and burned soils of the two west-central Oregon sites did not differ from

Table 2—The pH and carbon and nitrogen content of soils assayed for hydroxymate siderophore concentration (mean ± SE).†

Site	pH			Carbon			Nitrogen		
	Undisturbed	Logged-burned	Logged-unburned	Undisturbed	Logged-burned	Logged-unburned	Undisturbed	Logged-burned	Logged-unburned
				g/100 g					
Southwest 1, 2, 3	5.15 ± 0.27	6.13 ± 0.09	5.57 ± 0.21	5.40 ± 2.21	7.10 ± 0.51	6.69 ± 0.55	0.18 ± 0.03	0.18 ± 0.03	0.19 ± 0.02
West-central 1	5.52 ± 0.04	5.68 ± 0.11	5.68 ± 0.07	3.34 ± 0.24	4.84 ± 0.80	5.89 ± 0.56	0.16 ± 0.01	0.22 ± 0.01	0.24 ± 0.03
2	5.27 ± 0.03	5.10 ± 0.32	4.87 ± 0.03	3.18 ± 0.13	10.45 ± 1.03	4.02 ± 1.03	0.15 ± 0.01	0.32 ± 0.03	0.18 ± 0.03
East-central 1	5.53 ± 0.09	6.51 ± 0.10	–	3.09 ± 0.55	3.57 ± 0.51	–	0.11 ± 0.01	0.14 ± 0.02	–
2	5.57 ± 0.06	6.29 ± 0.32	–	3.08 ± 0.28	3.81 ± 0.59	–	0.10 ± 0.01	0.12 ± 0.01	–
3	5.73 ± 0.03	5.89 ± 0.09	–	2.11 ± 0.16	2.85 ± 0.07	–	0.08 ± 0.01	0.10 ± 0.02	–
4	5.56 ± 0.05	6.09 ± 0.17	5.80 ± 0.33	4.16 ± 0.48	3.33 ± 0.60	6.69 ± 0.55	0.16 ± 0.01	0.14 ± 0.02	–

† Standard errors are determined from three replicate field samples per site, except for southwest Oregon sites 1, 2, and 3, for which the mean refers to the average of the three sites, with one replicate per site.

Table 3—Hydroxymate-siderophore concentrations (± SE) in various disturbed and adjacent undisturbed forest soils in Oregon.*

Site	Concentration (μg/mL Desferal equivalent) Undisturbed	Logged-burned	Logged-burned
Southwest Oregon; 1, 2, 3	11.05a ± 2.14	1.45b ± 1.74	4.11b ± 1.18
4	1.62a ± 0.23	0.20b ± 0.19	–
West-central Oregon; 1	2.27a ± 0.37	1.45a ± 0.30	–
2	0.12a ± 0†	0.08a ± 0.04	0.25b ± 0.03
East-central Oregon; 1	0.19a ± 0	–	0.21a ± 0.47
2	1.09a ± 0.16	0.19b ± 0.13	–
3	0.87a ± 0.06	0.16b ± 0.05	–
4	1.18a ± 0.21	0.55b ± 0.17	1.10a ± 0.18

* Means in any one row that are followed by different letters are significantly different at the 0.05 level.
† Two replicates.

those in undisturbed soils. In the west-central site with logged and unburned soils, HS concentrations were higher than in undisturbed soils.

Both soil source (undisturbed or logged-broadcast-burned) and soil treatment (soil pasteurization, Fe^{3+} fertilization) had a significant effect on seedling weight. Trees grown in undisturbed forest soil had heavier tops than those grown in soil from the broadcast-burned area; however when seedlings were fertilized with Fe^{3+}, there was no difference (Fig. 1). Root weights did not differ between disturbed and undisturbed soils; however, as with tops, roots responded positively to Fe^{3+} fertilization in disturbed, but not in undisturbed, soils (Fig. 2). Pasteurization of undisturbed soil reduced seedling top weight—an effect which could be reversed by Fe^{3+} fertilization—but it did not affect roots. Pasteurization of logged and burned soils had the opposite effect: root weight was reduced and top weight was unaffected. Addition of Fe^{3+} to pasteurized soil affected tops and roots in the same ways; both responded positively in undisturbed soils and did not respond at all in disturbed soils.

Total root tips per seedling did not differ between disturbed and undisturbed soils; however, ectomycorrhizal formation was significantly lower in the logged and burned than in the undisturbed soil (Table 4).

Table 4—The effect of soil disturbance on the total number of root tips and the number of ecotmycorrhizal root tips formed on Douglas-fir seedlings (southwest Oregon site 3).

Soil and fertilization level	Total root tips	Ectomycorrhizal root tips per seedling
	$\bar{x}$ ± SE	
Undisturbed	116a ± 8	72a ± 8
Undisturbed + Fe^{3+}	115a ± 8	80a ± 7
Logged and burned	114a ± 9	46b ± 4
Logged and burned + Fe^{3+}	107a ± 12	34b ± 11

* In each column, different letters indicate that means are significantly different at the 0.05 level.

Neither the total number of root tips nor ectomycorrhizal tips were affected by Fe^{3+} fertilization. Seedlings grown in pasteurized soils, not included in the above analysis, formed ectomycorrhizae with *Thelephora terrestris*, a common greenouse contaminant.

DISCUSSION

Desorption of HS from soil reaches 70% of equilibrium within 1 h (Powell et al., 1982), so our 24-h extraction should have been sufficient to allow all tested soils to approach equilibrium desorption, and HS concentrations assayed should accurately reflect differences among soils. Our assay gives concentrations on a weight/volume basis rather than on a weight/weight basis, which can be misleading when soils with different bulk densities are compared; however, HS availability within a given rooting volume is more accurately reflected.

HS concentrations are strongly dependent on soil organic matter (Powell et al., 1982), and significant concentrations may occur in litter layers as well (S.L. Rose, unpublished data). Soil C was either unaffected or increased by logging on our study sites; therefore reduction in soil organic matter probably does not explain reduced HS concentrations in logged areas. However, on burned areas, litter layers were likely reduced, and an undetermined fraction of soil C was charcoal. Either factor may have affected HS concentrations.

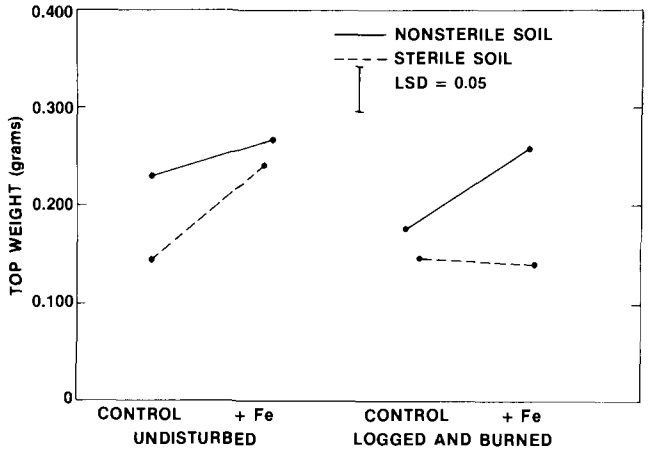

Fig. 1—Top weight of Douglas-fir seedlings grown in undisturbed and disturbed soils, with and without Fe fertilization and soil sterilization. Least significant difference (LSD) at the 0.05 probability level.

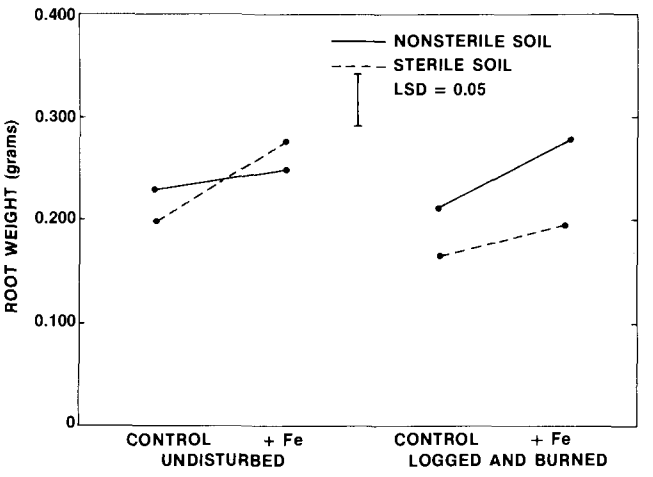

Fig. 2—Root weight of Douglas-fir seedlings grown in undisturbed and disturbed soils, with and without iron fertilization and soil sterilization. Least significant difference (LSD) at the 0.05 probability level.

Like C, soil N was either unaffected or increased by logging on our sites. Because N volatilization during fire is closely correlated with heat of the burn (Debano et al., 1979), it appears that low-intensity slash burns were the rule on these sites. However, in previous work on southwest Oregon sites 1, 2, and 3, we found that logged and burned portions had sharply higher ratios of bacteria to fungi than did logged and unburned or undisturbed areas (Perry and Rose, 1983), and this study shows that mycorrhizal formation is reduced in logged and burned soils of site 3. Thus, the level of disturbance was sufficient to alter the soil biological community on these sites. Since HS producers are primarily fungi, including ectomycorrhizae (Szaniszlo et al., 1981), this alteration may account for HS reduction. Because many HS producers are rhizosphere organisms, removal of living roots may also lead to decreased HS production. Powell et al. (1980) found a positive correlation between soil HS concentrations and root density.

Our seedling bioassay indicates that, on the site we tested, growth in broadcast-burned soil is limited either directly or indirectly by chelated iron. This is not surprising, as Fe^{3+} is much less soluble at the pH of the broadcast-burned soil (6.16) than at the pH of the undisturbed soil (4.68), and as we detected virtually no HS in the broadcast-burned soil of this particular site. Because burning generally raises the pH of forest soils, HS concentrations may be particularly important in Fe nutrition of seedlings in broadcast-burned areas. Naturally occurring organic acids, which are thought to be important in the Fe nutrition of many higher plants, are ineffective as Fe chelators at pH much above 6, while HS–Fe complexes are stable up to pH 10 (Powell et al., 1982; Cline et al., 1982).

Reviewing the literature, Powell et al. (1982) conclude that HS are important in the Fe nutrition of higher plants. They suggest that, even with high HS concentrations in soils, zones of depletion may form in rhizospheres because of low HS mobility. Consequently, HS availability to higher plants may be controlled more by production by rhizosphere organisms than by soil storage capacity. That adding Fe in our pot study reversed the reduction of seedling growth after sterilization of undisturbed soils supports the hypothesis that microbial production controls HS availability—at least in some cases. It is notable that the response of both tops and roots to added Fe^{3+} in nonsterile, burned soils was similar to the Fe^{3+} response in sterile, undisturbed soils, suggesting that HS-producing organisms were reduced or eliminated by logging and broadcast burning on this site. Whatever the effect, it had persisted for 10 years.

Unlike seedlings grown in sterile, undisturbed soil, those grown in sterile broadcast-burned soil did not respond to chelated Fe. This may be related to contamination of sterile soils by *T. terrestris* (although undisturbed sterile soils were contaminated also) or may indicate some other weakness in our experimental technique. Alternatively, seedling growth in the burned soil may be limited indirectly, rather than directly, by chelated Fe—if, for example, N-mineralizing organisms were Fe limited and their inactivity in turn limited seedling growth. In sterile soils, added Fe would then have no effect on seedling growth. We could not visually detect symptoms of Fe deficiency in seedlings (foliage was not analyzed), which suggests indirect limitation. The results of the seedling bioassay should be considered preliminary until repeated and extended to other sites.

Kloepper et al. (1980) hypothesized that certain rhizobacteria serve a protective function for higher plants because the siderophore that they produce binds Fe too tightly to be available to pathogens (similar Fe chelators perform this function in mammalian blood). We have found sharply reduced HS concentrations at the active front of one large *Phellinus weirii* (laminated root rot) infection in an Oregon mountain hemlock stand (Rose, Cromack, and Perry, unpublished data). This may be an effect of the root rot (through generally reduced activity) rather than a cause; however, we should not rule out the possibility that lower HS concentrations reduce tree resistance to the pathogen.

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